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Responsivity to PGE2 labor induction involves concomitant differential prostaglandin E receptor gene expression in cervix and myometrium

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ABSTRACT. Prostaglandin E2 (dinoprostone) is largely used for labor induction. However, one-third of patients do not respond to treatment. One cause of this poor response may be associated with changes in regulation of prostaglandin E receptors (EP1-4). In this study, we investigated EP mRNA expression in the uterine cervix and lower uterine segment myometrium for term births. Biopsies were obtained from women with successful (responders) and failed

(non-responders) dinoprostone labor induction, while women that underwent spontaneous labor were included as controls. EP1 mRNA was upregulated in the cervical tissue of women who did not respond to dinoprostone induction. In addition, in the myometrium, significantly higher levels of EP3 mRNA were observed in women treated with dinoprostone, independent of their responsiveness. Dinoprostone-responders presented 3.6-fold higher levels of EP3 mRNA expression than the spontaneous labor group. Significantly higher levels of EP3 mRNA in the myometrium of the dinoprostone-treated group indicated that dinoprostone may regulate the EP3 gene on the transcriptional level. These results highlight the relationship between EP gene expression and delivery and indicate that understanding the regulation of prostaglandin E receptors may lead to improved labor induction.

Key words: Dinoprostone; mRNA; Prostaglandin receptors; Uterus; Term pregnancy

INTRODUCTION

Human birth is a physiological event triggered by inflammatory molecules, mainly prostaglandins (PGs) and cytokines (Olson, 2003; Mittal et al., 2010; Hua et al., 2012). Among these, PGE2 (dinoprostone) plays a central role in parturition, contributing to uterine contractility, membrane rupture, and cervical ripening (Olson et al., 2003). PGE2 acts by signal transduction through the 7-transmembrane domain G-protein-coupled EP receptors, which are classified into 4 subtypes: EP1, EP2, EP3, and EP4 (Coleman et al., 1994; Narumiya et al., 1999; Woodward et al., 2011; Sugimoto et al., 2015); the gene symbols for these receptors are *PTGER1*-*PTGER4* (Gu et al., 2012).

The 4 PGE2 receptor subtypes are found in the human uterine myometrium and cervix (Smith et al., 1998, 2001; Myatt and Lye, 2004; Astle et al., 2005), as well as in the amnion, choriodecidua, and placenta (Grigsby et al., 2006a; Unlugedik et al., 2010). PGs released from these tissues stimulate uterine contractility (Hertelendy and Zakar, 2004) and cervical ripening (Schmitz et al., 2003; Roos et al., 2014), suggesting paracrine and autocrine roles for PGE2 in the signaling pathways associated with human parturition (Schmitz et al., 2003; Grigsby et al., 2006a). Previous studies have suggested that myometrial activation (from quiescence during pregnancy to contractile activity at time of labor) may be directly modulated by combination or balance of EP receptor expression between maternal and fetal tissues, both over the course of pregnancy and during parturition (Astle et al., 2005; Grigsby et al., 2006b).

Because of the important role of dinoprostone at birth in cervical ripening and myometrial contractile activity (Chioss et al., 2012), it is used pharmacologically for labor induction in women presenting an unfavorable cervix. A recent meta-analysis performed by Austin et al. (2010) suggested that dinoprostone showed satisfactory efficacy and safety in labor induction. However, approximately 1/3 patients fail to respond to dinoprostone labor induction (Tenore, 2003). The main factors involved with unresponsiveness to dinoprostone are not fully understood. We hypothesized that failure of dinoprostone labor induction is related to differential gene regulation of EP receptors in the cervical and myometrial tissues between responsive and nonresponsive women.

We performed this case-control study to evaluate the mRNA expression of the EP1, EP2, EP3, and EP4 genes in the uterine tissue of term pregnant women responsive/nonresponsive to dinoprostone treatment.

MATERIAL AND METHODS

Subjects

The study included 30 term pregnant women, who were divided into 3 groups: spontaneous labor (SL), which was the positive control group and included women presenting natural labor initiation followed by vaginal delivery or cesarean after spontaneous labor; dinoprostone responder group (DR), which included women treated with dinoprostone, presenting effective uterine contractions, cervical effacement, and dilatation, who progressed to vaginal delivery or cesarean after the dinoprostone response; dinoprostone nonresponder group (DNR), which included women without uterine contraction or cervical effacement and failure in labor induction after dinoprostone administration.

Term was considered as pregnancy between 37 and 41 + 6 weeks of gestational age and spontaneous labor as natural labor initiation. Patients with indication for labor induction and with an unripe cervix, defined as a Bishop's score of ≤5 points (for Bishop score see Table 1 in Tenore, 2003), were assigned to receive 10 mg vaginally administered dinoprostone (Propess®, Ferring Pharmaceuticals, São Paulo, SP, Brazil) placed in the posterior vaginal fornix as described previously (Tan et al., 2009). Bishop 1 was considered the Bishop score upon admission to labor and Bishop 2 was immediately before birth. Labor induction failure was considered to be the absence of uterine contractions and no cervical ripening over 24 h (Pevzner et al., 2009).

Pregnant women with the following characteristics were excluded from the study: smokers and those with other drug abuse; women with a disease or undergoing chronic use of drugs that may influence prostaglandin metabolism, and preterm or post-term pregnancies. Women with infections and premature rupture of membranes were also excluded from the study.

Tissue collection

Cervical and myometrial tissue biopsies were obtained according previously described protocols (Stjernholm-Vladic et al., 2004; Astle et al., 2005) from women with term pregnancies after vaginal delivery (cervix) or during/after cesarean section (myometrium and cervix). Myometrium biopsies were taken from the lower edge of the upper part of the incision in the lower uterine segment during cesarean, after placenta removal. Cervix biopsies were taken from the anterior labia after placenta removal in vaginal labors or immediately after cesarean. After excision, tissues were immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

EP receptor mRNA expression analysis, RNA extraction, reverse transcription, and real-time polymerase chain reaction

To analyze the differential gene expression of EP receptors among groups, RNA was extracted from uterine cervical and myometrial cells using Trizol (Life Technologies, Carlsbad,

CA, USA) according to the manufacturer instructions. Quantitation and estimation of total RNA purity was performed using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA; absorbance ratio of 260/280 nm) spectrophotometer. Ratios above 1.8 were considered pure, and samples below this threshold were discarded. Complementary DNA was synthesized from 1 μg total RNA, which was first treated with 0.1 U amplification-grade DNase (Life Technologies) for 5 min at 37°C. After DNase inactivation at 65°C for 10 min, samples were incubated in a final volume of 20 μL with 1 μM oligo-dT primer, 4 U omniscriptRTase (Omniscript RT Kit, Qiagen, Hilden, Germany), 0.5 mM dNTPs (Invitrogen, Carlsbad, CA, USA), and 10 U RNase inhibitor (Invitrogen).

Quantitative polymerase chain reactions were conducted in a CFX384 thermocycler (Bio-Rad, Hercules, CA, USA) using iQ SYBR Green Supermix (Bio-Rad) and human-specific primers (Table 1) designed using the Primer Express Software (Applied Biosystems, Foster City, CA, USA). Melting-curve analyses were performed to verify product identity. To optimize the quantitative polymerase chain reaction assay, serial dilutions of cDNA templates were used to generate a standard curve. A standard curve was constructed by plotting the log of the starting quantity of the dilution factor against the Ct value obtained during amplification of each dilution. Reactions with a coefficient of determination (R^2) higher than 0.98 and efficiency from 95-105% were considered to be optimized. The relative standard curve method was used to assess the amount of transcript in each sample (Cikos et al., 2007). Samples were run in duplicate and the results were expressed relative to the average Ct values for β-actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal controls. Internal control genes were selected based on Ct variance (as reflected by the standard deviation) among the groups in each experiment.

Table 1. List of primers used during prostaglandin receptor gene expression experiments.

Gene	Forward primer	Reverse primer	GenBank or Ensembl accession No.
PTGER1	ATGGTGGGCCAGCTTGTC	GCCACCAACACCAGCATTG	NM_000955.2
PTGER2	GAAACCTCTTCCCGAAAGGAA	AATCCGACAACAGAGGACTGAAC	NM_000956.3
PTGER3	AGCTTATGGGGATCATGTGC	TTTCTGCTTCTCCGTGTGTG	NM_198715.2
PTGER4	GAAAGCTGGCAACTCTGACC	GCTTTCACCTTGTCCTGCTC	NM_000958.2
GAPDH	GTCGGAGTCAACGGATT	GTCGGAGTCAACGGATT	NM_002046.4
β-actin	TGTGGATCAGCAAGCAGGAGTA	TGCGCAAGTTAGGTTTTGTCA	NM_001101.3

Statistical analysis

Data were analyzed using the SPSS statistical software version 19.0 (SPSS, Inc., Chicago, IL, USA) and the results are reported as means ± SD or median conforming analysis. Baseline data were compared among groups by one-way analysis of variance followed by the Bonferroni *post-hoc* test. mRNA expression was compared among groups using non-parametric Kruskall-Wallis analysis of variance followed by the Mann-Whitney *post-hoc* test or the median test. To evaluate potential intervenient variables on mRNA expression, the Spearman correlation test was also performed. All tests were 2-tail analyzed and P values ≤ 0.05 were considered to be significant.

Ethical approval

The present study, involving human cervical and myometrial tissue biopsies at the

time of parturition, was approved by Universidade Federal de Santa Maria Ethical Committee (CAAE 0268.0.243.000-08), and informed consent was obtained from all participants before enrollment.

RESULTS

Clinical baselines

A total of 30 term pregnant women were included in the present study. More than half (18) of the patients (60%) had indication for labor induction due to gestational age greater than 41 weeks (41 weeks to 41 + 6 weeks) or uncomplicated hypertension. Twelve of these (66.7%) responded to dinoprostone induction and underwent vaginal labor or cesarean (due to dystocia, fetal distress, or hypertonia) and 6 (33.3%) failed in induction (delivered by cesarean). Twelve patients of the total (40%), considered to be the control group, entered the study with spontaneous labor and underwent vaginal labor or cesarean (due to dystocia or fetal distress). Baseline characteristics of subjects studied are described in Table 2. Mean maternal age, gestational age, and parity were similar among groups. Time for dinoprostone-induction labors was longer than for spontaneous labors, regardless of the delivery route. Bishop scores showed a significant difference between groups over time. Patients beginning labor spontaneously showed higher initial Bishop scores than patients with labor induction indication. Bishop scores increased significantly in all groups over time. Interestingly, although patients considered to be non-responders to dinoprostone induction showed increased Bishop scores over time, their final Bishop scores still represented immature cervices (Bishop ≤ 5). The significant differences between the duration of labor and Bishop scores were inherent to the clinical characteristics of each group.

Table 2. Baseline subject characteristics.

Variable	Groups			P
	SL (N = 12)	DR (N = 12)	DNR (N = 6)	
Age (years)	23.7 ± 1.46[a]	23.7 ± 1.13[a]	24.8 ± 2.39[a]	0.8447
Gestational age (weeks)	39.4 ± 0.41[a]	39.3 ± 0.41[a]	40.0 ± 0.59[a]	0.7861
Time of labor (h)	8.42 ± 2.33[a]	18.1 ± 1.91[b]	23.3 ± 1.41[b]	<0.0001
Bishop 1	5.83 ± 0.63[a]	3.5 ± 0.26[b]	2.17 ± 0.17[b]	<0.0001
Bishop 2	8.42 ± 0.94[a]	9.83 ± 0.46[a]	3.83 ± 0.60[b]	<0.0001

SL = spontaneous labor (positive control); DR = dinoprostone responder; DNR = dinoprostone non-responder. Bishop 1 = Bishop score at admission to labor. Bishop 2 = Bishop score immediately before birth. Data are reported as means ± SD. Different letters indicate significant differences by parametric one-way analysis of variance followed by the Bonferroni *post-hoc* test. Variables with $P < 0.05$ were considered to be significant.

EP receptor mRNA expression (cervix and myometrium)

The differential mRNA expression of EP receptors (*PTGER1*, *PTGER2*, *PTGER3*, and *PTGER4*) in relation to β-actin and GAPDH mRNA was investigated in uterine tissues. These results are presented as relative EP mRNA expression relative to β-actin and GAPDH. The association among differential EP mRNA expression in the cervical and myometrial tissues and spontaneous or dinoprostone-induced labor were evaluated. Because EP mRNA

expression did not show a normal distribution, data were analyzed using non-parametric statistical tests and described as the median (Table 3). EP gene expression was described as downregulated or upregulated compared to β-actin and GAPDH gene expression (Figure 1).

Table 3. Relative mRNA expression of prostaglandin receptors in uterine tissues of women with spontaneous and dinoprostone-induced labors.

Gene	Uterine tissues	Groups			P
		SL	DR	DNR	
EP1	Cervix	0.01	1.06	1.53	0.024
	Myometrium	4.38	0.01	2.35	0.749
EP2	Cervix	1.88	0.16	2.96	0.092
	Myometrium	1.51	2.68	1.55	0.476
EP3	Cervix	0.01	0.05	0.08	0.792
	Myometrium	4.44	16.19	6.25	0.034
EP4	Cervix	22.09	0.63	3.17	0.236
	Myometrium	16.92	0.01	7.27	0.167

SL = spontaneous labor (positive control); DR= dinoprostone responder; DNR= dinoprostone non-responder. Values represent median value of relative mRNA expression of prostaglandin receptors (EPs) in relation to β-actin and GAPDH mRNA. P values correspond to statistical comparison among groups by the Kruskall-Wallis test followed by the Man-Whitney *post-hoc* test.

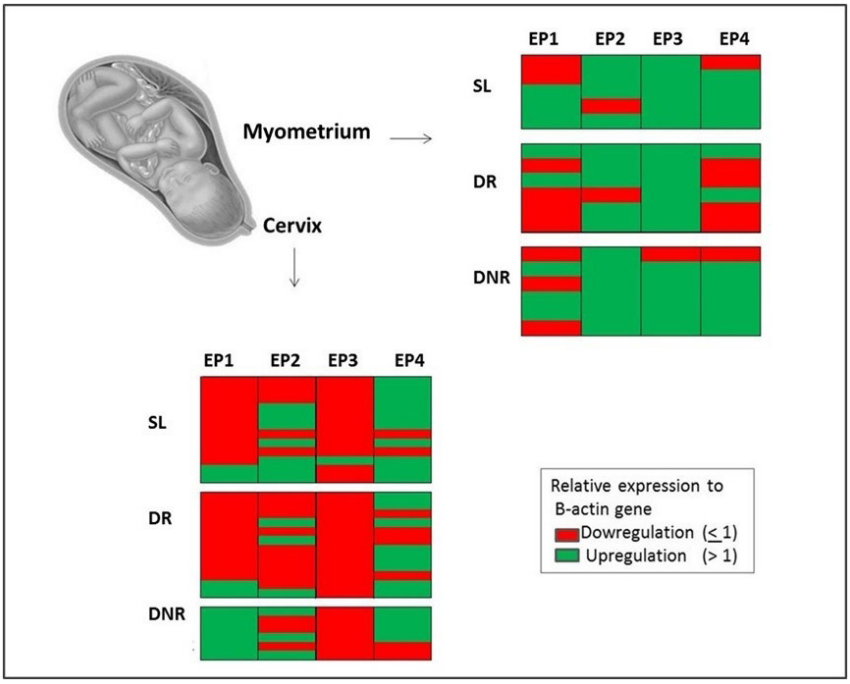

Figure 1. Schematic representation of prostaglandin receptors (EP1, EP2, EP3, and EP4) gene expression in myometrial and cervical tissues of term women with spontaneous labor (SL), dinoprostone-induction responders (DR), and dinoprostone-induction non-responders (DNR). EP mRNA was quantificated relative to the expression of the β-actin and GAPDH constitutive genes and grouped into the following categories: dowregulation (≤1 times of β-actin and GAPDH expression) and upregulation (>1 times of β-actin and GAPDH expression). Line represents the expression of genes from each patient examined.

In the cervical tissue, women in the SL group showed EP1 downregulation in most cases; similar results were observed in DR women. However, 100% (N = 6) of DNR women showed EP1 mRNA upregulated in relation to β-actin and GAPDH mRNA expression (P = 0.024). The chance of DNR group to present gene upregulated was 2.5-fold higher (95% confidence interval = 1.170-4.341) than in the SL and DR groups.

Based on the significant association between EP1 relative mRNA expression in the cervical tissue and dinoprostone responsiveness, the correlation between EP1 mRNA expression and variables related to delivery was evaluated. As shown in Table 4, EP1 mRNA expression in SL women was not associated with any delivery variable investigated. However, DR women presented a significant inverse correlation between EP1 mRNA expression and gestational age and also Bishop 2 score. A greater gestational age (P = 0.037) and greater Bishop 2 score (P = 0.010) were associated with a higher dinoprostone response and EP1 downregulation in the cervix. The EP3 gene in cervical tissue was downregulated in the SL group and in women treated with dinoprostone, independently of the labor induction response (Table 3). Therefore, this gene did not show differential expression among groups (P = 0.792). EP2 and EP4 gene expression was highly variable, but there was no significant difference among groups (EP2 P = 0.092; EP4 P = 0.792).

Table 4. Correlation among EP1 gene expression compared to β-actin and the GAPDH gene in cervix tissue and variables related to delivery.

Variables	SL EP1 gene		DR EP1 gene		DNR EP1 gene	
	r	P	r	P	r	P
Maternal age	0.340	0.306	0.271	0.393	0.174	0.742
Gestation age	-0.049	0.901	-0.065	0.037	0.429	0.397
Time induction	0.459	0.156	0.381	0.247	-0.116	0.827
Bishop 1	0.166	0.626	0.010	0.999	-0.655	0.158
Bishop 2	0.130	0.970	-0.705	0.010	-0.309	0.552

SL = spontaneous labor; DR = dinoprostone responder; DNR = dinoprostone non-responder; r = Spearman correlation coefficient; P = significant value.

In the analysis of EP mRNA in the myometrium, as shown in Table 3, we observed similar mRNA expression patterns among all groups compared to EP1 (P = 0.749), EP2 (P = 0.476), and EP4 (P = 0.167). Although most samples showed upregulation of the EP3 gene independently of the group, significantly higher expression levels of EP3 mRNA were observed in women treated with dinoprostone, independently of responsiveness, when compared to the SL group (P = 0.034). In the myometrial tissue, the DR group showed 3.6-fold higher levels of EP3 mRNA expression than did the SL group, indicating that dinoprostone regulates the EP3 gene at the transcriptional level.

DISCUSSION

The present study described the potential association between differential EP gene expression in the cervix and myometrium and dinoprostone response in term women. In this study, we examined the gene regulation of physiological EPs in myometrial and cervical tissues in spontaneous labor and with respect to pharmacological dinoprostone action on these genes.

Prostaglandins play a central role in human labor, leading to cervical ripening and myometrial contractility (Schmitz et al., 2003; Hertelendy and Zakar, 2004; Roos et al., 2014). Classically, myometrium tissue analyses have found that PGE2 stimulates contractions via EP1 and EP3 receptors, whereas EP2 and EP4 maintain quiescence (Brodt-Eppley and Myatt, 1999). However, few studies have been examined these concepts in detail (Astle et al., 2005; Grigsby et al., 2006a; Arulkumaran et al., 2012; Kandola et al., 2014), and results are inconsistent. This is the first study to observe concomitant and antagonic EP1 and EP3 mRNA expression regulation in the cervical and myometrial tissues in term women with spontaneous labor, indicating differential uterine tissue-regulation during physiological parturition.

EP1 and EP3 are contractile receptors, but act in different manners. EP1 mediates the elevation of intracellular Ca^{2+} concentration, while EP3, which is considered to be an inhibitory receptor, inhibits adenylate cyclase via inhibitory G protein, mediates decreased cyclic adenosine monophosphate levels, and inhibits smooth muscle relaxation. However, EP2 and EP4 are classified as relaxant receptors, which couple to G proteins to stimulate adenylate cyclase and induce smooth muscle relaxation by elevating cyclic adenosine monophosphate production (Narumiya et al., 1999; Sugimoto and Narumiya, 2007). Myometrium upregulation of EP3 gene expression described here agree with the results of previous studies reporting that EP3 is likely the primary receptor subtype mediating PGE2-stimulated myometrial contractions (Arulkumaran et al., 2012). These authors concluded that EP3 is likely mainly contractile in its action, remaining on the cell surface and can bind to extracellular exogenous PGE2.

In functional terms, because parturition involves a shift from uterine quiescence to progressive myometrium contractility, labor is expected to be accompanied by an increase in contractile PGE2 receptors and a decrease in relaxatory PGE2 receptors in myometrium tissues. Because myometrial quiescence is promoted by increased cyclic adenosine monophosphate (Yuan and López-Bernal, 2007), an increase in the expression of genes such as EP3 in the myometrium is expected during physiological parturition, as was observed in our results. This finding has been corroborated by other studies (Arulkumaran et al., 2012), which mainly correlate EP3 with myometrial contractile function.

However, why EP3 is downregulated in the cervix during spontaneous labor (Table 3) is not well understood. The cervix is a rigid structure during most times in a woman's life, except near parturition and during labor, when this region loses competence to permit delivery. Cervix competence loss involves coordinated and complex biochemical and physiological mechanisms, in which the extracellular matrix, consisting of collagen, hyaluronan, proteoglycans, and immune components, is transformed. This cervical remodeling consists of 4 phases, including softening, ripening, dilation, and postpartum repair (Timmons et al., 2010).

Additionally, a recent investigation using rats as an experimental model described that the cervix is also composed of smooth muscle cells (Ferland et al., 2015). This also appears in humans, and smooth muscle contractility was described in human tissues 30 years ago (Norstrom et al., 1984). Thus, as described by Ferland et al. (2015) using electromyography and pharmacology evidence, cervix and uterus can be considered as separate organs in functional terms. Thus, smooth muscle cells in the cervix may play an active role in cervical remodeling during pregnancy and parturition, which would explain the occurrence of differential EP3 gene regulation in this tissue compared to in the myometrium.

Studies of cervical biopsies obtained from post-term women showed that failure of labor induction may be caused by increased expression of EP3 and a concomitant decrease in EP4 expression compared with spontaneous labor (Roos et al., 2014). Although it is not

possible to compare our results with this study because the subject were different, regarding the gestational age at labor induction moment (post-term *vs* term), both studies found lower EP3 expression in the cervical tissue in spontaneous labor, indicating the relevance receptor gene regulation in human parturition.

Interestingly, EP1 mRNA was also downregulated in the cervix of women presenting spontaneous labor. Modulation of the EP1 gene in cervical tissues during labor has not been thoroughly examined. Our findings show clearly that this gene was downregulated in the cervix and upregulated in the myometrium of women with spontaneous labor (Table 3). In addition, EP1 played an important role related to the dinoprostone response in labor induction.

Moreover, a recent study in mice suggested that prostaglandins do not control all aspects of parturition (Timmons et al., 2014). The study provided evidence for 2 distinct pathways of cervical ripening: one dependent on PGs derived from paracrine or endocrine sources, and another independent of PG actions. Therefore, complementary and independent studies should be performed to confirm whether differential EP gene expression in the cervical and myometrial tissues represents a universal physiological response related to human parturition.

We also examined the association between EP gene expression and dinoprostone responsivity. Up to 30-40% of obstetrical patients require labor induction using pharmacological drugs including oxytocin and analogs of prostaglandins E1 and E2. These drugs are particularly useful when labor induction necessitates cervical ripening, as when labor induction occurs in an unfavorable cervix (Hawkins and Wing, 2012). However, not all women respond to induction, and factors leading to labor failure must be further examined. We hypothesized that altered EP gene expression in the myometrium and cervix were related to dinoprostone responsiveness.

Our results suggest that dinoprostone acts on the transcriptional level, affecting the EP1 and EP3 genes. EP3 mRNA was downregulated in the cervical tissue of most patients, independently of the case-control groups (Figure 1). In the myometrium of women treated with dinoprostone, increased expression of EP3 mRNA was observed compared to in the SL group. Because EP3 upregulation occurred in both the responsive and nonresponsive dinoprostone groups, we hypothesized that the effect on this gene was not the main causal factor triggering labor induction success, despite the higher EP3 levels observed in the DR group. Because EP3 expression is associated with myometrium contractility, increased EP3 mRNA expression in the DNR group indicated that failure in labor probably likely resulted from post-transcriptional regulation of EP3 genes or other biochemical factors not analyzed in this study.

In contrast, EP1 mRNA gene expression differed between the DR and DNR groups in the cervical tissue. In the SL and DR groups, we observed that EP1 mRNA was downregulated, whereas in the DNR group, this gene was upregulated. Similar results were not observed in previous studies. The difference in EP1 gene regulation in the cervix in dinoprostone responsive and nonresponsive women indicates its relevance in spontaneous and pharmacologically induced parturition.

The strength of this study is the concomitant analysis in cervical and myometrial tissues of the effect of prostaglandin receptors on gene expression (*PTGER1-PTGER4*) during the labor induction response. Most studies conducted isolated analyses of uterine tissues, limiting the visualization of integrated response effects.

There were some limitations to our study. The number of subjects was low. Despite the high number of deliveries in our hospital, it is difficult to select patients to participate in such studies for several reasons, including high diversity of physiological and pathological

conditions and the clinical and pharmacological indication of use of drugs such as dinoprostone.

Another potential limitation is related to the fact that we only investigated the EP mRNA expression and not the EP protein expression. Protein expression of these receptors may be relevant. However, because EP transcriptional modulation precedes protein expression, we investigated differential EP transcriptional modulation in dinoprostone-induced labor. There may be differences in the uterine tissues of pregnant women regarding the physiological aspects of late pregnancy, and it would be more difficult to determine whether protein expression was related to a pharmacological response.

It would be ideal to biopsy the same patient before, during, and after labor to determine the differences in EP gene and protein regulation in response to dinoprostone. However, this practice is not ethically acceptable. Thus, an alternative strategy for confirming the dinoprostone effect on the EP1 and EP3 genes may include complementary *in vitro* studies using myometrial and cervical cellular culture.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Arulkumaran S, Kandola MK, Hoffman B, Hanyaloglu AC, et al. (2012). The roles of prostaglandin EP 1 and 3 receptors in the control of human myometrial contractility. *J. Clin. Endocrinol. Metab*. 97: 489-498.

Astle S, Thornton S and Slater DM (2005). Identification and localization of prostaglandin E2 receptors in upper and lower segment human myometrium during pregnancy. *Mol. Hum. Reprod.* 11: 279-287.

Austin SC, Sanchez-Ramos L and Adair CD (2010). Labor induction with intravaginal misoprostol compared with the dinoprostone vaginal insert: a systematic review and metaanalysis. *Am. J. Obstet. Gynecol*. 202: 624.e1-9.

Brodt-Eppley J and Myatt L (1999). Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet. Gynecol.* 93: 89-93.

Chioss G, Costantine MM, Bytautiene E, Betancourt A, et al. (2012). *In vitro* myometrial contractility profiles of different pharmacological agents used for induction of labor. *Am. J. Perinatol*. 29: 699-704.

Cikos S, Bukovska A and Koppel J (2007). Relative quantification of mRNA: comparison of methods currently used for real-time PCR data analysis. *BMC Mol. Biol.* 8: 113.

Coleman RA, Smith WL and Narumiya S (1994). International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* 46: 205-229.

Ferland DJ, Darios ES and Watts SW (2015). The persistence of active smooth muscle in the female rat cervix through pregnancy. *Am. J. Obstet. Gynecol*. 212: 244.e1-8.

Grigsby PL, Sooranna SR, Brockman DE, Johnson MR, et al. (2006a). Localization and expression of prostaglandin E2 receptors in human placenta and corresponding fetal membranes with labor. *Am. J. Obstet. Gynecol*. 195: 260-269.

Grigsby PL, Sooranna SR, Adu-Amankwa B, Pitzer B, et al. (2006b). Regional expression of prostaglandin E2 and F2alpha receptors in human myometrium, amnion, and choriodecidua with advancing gestation and labor. *Biol. Reprod*. 75: 297-305.

Gu G, Gao Q, Yuan X, Huang L, et al. (2012). Immunolocalization of adipocytes and prostaglandin E2 and its four receptor

proteins EP1, EP2, EP3, and EP4 in the caprine cervix during spontaneous term labor. *Biol. Reprod*. 86: 159, 1-10.
Hertelendy F and Zakar T (2004). Regulation of myometrial smooth muscle functions. *Curr. Pharm. Des*. 10: 2499-2517.
Hawkins JS and Wing DA (2012). Current pharmacotherapy options for labor induction. *Expert Opin. Pharmacother*. 13: 2005-2014.
Hua R, Pease JE, Sooranna SR, Viney JM, et al. (2012). Stretch and inflammatory cytokines drive myometrial chemokine expression via NF-kappaB activation. *Endocrinology* 153: 481-491.
Kandola MK, Sykes L, Lee YS, Johnson MR, et al. (2014). EP2 receptor activates dual G protein signaling pathways that mediate contrasting proinflammatory and relaxatory responses in term pregnant human myometrium. *Endocrinology* 155: 605-617.
Mittal P, Romero R, Tarca AL, Gonzalez J, et al. (2010). Characterization of the myometrial transcriptome and biological pathways of spontaneous human labor at term. *J. Perinat. Med*. 38: 617-643.
Myatt L and Lye SJ (2004). Expression, localization and function of prostaglandin receptors in myometrium. *Prostaglandins Leukot. Essent. Fatty Acids* 70: 137-148.
Narumiya S, Sugimoto Y and Ushikubi F (1999). Prostanoid receptors: structures, properties, and functions. *Physiol. Rev*. 79: 1193-1226.
Norstrom A, Bryman I, Wiqvist N, Sahni S, et al. (1984). Inhibitory action of relaxin on human cervical smooth muscle. *J. Clin. Endocrinol. Metab*. 59: 379-382.
Olson DM (2003). The role of prostaglandins in the initiation of parturition. *Best Pract. Res. Clin. Obstet. Gynaecol*. 17: 717-730.
Pevzner L, Rayburn WF, Rumney P and Wing DA (2009). Factors predicting successful labor induction with dinoprostone and misoprostol vaginal inserts. *Obstet. Gynecol.* 114: 261-267.
Roos N, Blesson CS, Stephansson O, Masironi B, et al. (2014). The expression of prostaglandin receptors EP3 and EP4 in human cervix in post-term pregnancy differs between failed and successful labor induction. *Acta Obstet. Gynecol. Scand*. 93: 159-167.
Schmitz T, Leroy MJ, Dallot E, Breuiller-Fouche M, et al. (2003). Interleukin-1beta induces glycosaminoglycan synthesis via the prostaglandin E2 pathway in cultured human cervical fibroblasts. *Mol. Hum. Reprod.* 9: 1-8.
Smith GC, Baguma-Nibasheka M, Wu WX and Nathanielsz PW (1998). Regional variations in contractile responses to prostaglandins and prostanoid receptor messenger ribonucleic acid in pregnant baboon uterus. *Am. J. Obstet. Gynecol*. 179: 1545-1552.
Smith GC, Wu WX and Nathanielsz PW (2001). Expression of prostanoid receptor genes in baboon chorion and decidua during pregnancy and parturition. *J. Endocrinol.* 168: 263-272.
Stjernholm-Vladic Y, Stygar D, Mansson C, Masironi B, et al. (2004). Factors involved in the inflammatory events of cervical ripening in humans. *Reprod. Biol. Endocrinol*. 2: 74.
Sugimoto Y and Narumiya S (2007). Prostaglandin E receptors. *J. Biol. Chem*. 282: 11613-11617.
SugimotoY, Inazumi T and Tsuchiya S (2015). Roles ofprostaglandin receptorsin female reproduction. *J. Biochem.*157: 73-80.
Tan PC, Daud SA and Omar SZ (2009). Concurrent dinoprostone and oxytocin for labor induction in term premature rupture of membranes: a randomized controlled trial. *Obstet. Gynecol*. 113: 1059-1065.
Tenore JL (2003). Methods for cervical ripening and induction of labor. *Am. Fam. Physician* 67: 2123-2128.
Timmons B, Akins M and Mahendroo M (2010). Cervical remodeling during pregnancy and parturition. *Trends Endocrinol. Metab*. 21: 353-361.
Timmons BC, Reese J, Socrate S, Ehinger N, et al. (2014). Prostaglandins are essential for cervical ripening in LPS-mediated preterm birth but not term or antiprogestin-driven preterm ripening. *Endocrinology* 155: 287-298.
Unlugedik E, Alfaidy N, Holloway A, Lye S, et al. (2010). Expression and regulation of prostaglandin receptors in the human placenta and fetal membranes at term and preterm. *Reprod. Fertil. Dev.* 22: 796-807.
Woodward DF, Jones RL and Narumiya S (2011). International Union of Basic and Clinical Pharmacology. LXXXIII: classification of prostanoid receptors, updating 15 years of progress. *Pharmacol. Rev*. 63: 471-538.
Yuan W and López-Bernal A (2007). Cyclic AMP signalling pathways in the regulation of uterine relaxation. *BMC Pregnancy Childbirth* 7 (Suppl 1): S10.